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ORIGINAL ARTICLE

A New Species of *Gracixalus* (Anura: Rhacophoridae) from West Guangxi, China

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Abstract We discovered a new species of the genus *Gracixalus*, *Gracixalus tianlinensis* sp. nov. which is morphologically almost similar to *G. jinggangensis*, *G. jinxiuensis* and *G. sapaensis*, but is distinguished from these species and all other rhacophorids in China and adjoining countries by a combination of the following characters: (1) SVL 30.3-35.9 mm in male, 35.6-38.7 mm in female, (2) head length less than head width, (3) vomerine teeth absent, (4) supratympanic fold distinct, (5) axilla and posterior surface of flanks pale yellow, (6) nuptial pads distinct on Finger I and slightly visible on Finger II, (7) dorsum brown to beige, with an inverse Y-shaped dark brown marking, (8) single subgular vocal sac. Our preliminary phylogenetic analyses implied *G. tianlinensis* sp. nov. is sister to *G. sapaensis* with well-supported values. Currently, this new species is known to be distributed in montane evergreen forests in association with montane bamboo in Cenwanglaoshan National Nature Reserve, Tianlin County, Guangxi, China.

Keywords *Gracixalus tianlinensis* sp. nov., Rhacophoridae, southern China

1. Introduction

The genus *Gracixalus* was originally treated as a subgenus of *Aquixalus* (Delorme *et al.*, 2005). Li *et al.* (2008, 2009), however, suggested assigning *Gracixalus* to a distinct genus based on their phylogenetic analyses and treated *Gracixalus gracilipes* as the type species of the genus. Currently, the species of *Gracixalus* occurs in China, Vietnam, Laos, Thailand and Myanmar. Till date, the genus *Gracixalus* contains 13 recognized species, and most of them are described recently (Frost, 2017). However, the specific composition of the genus *Gracixalus* is still unknown. For instance, the group of *Gracixalus jingxiuensis* contains cryptic species that are primarily based upon preliminary mitochondrial DNA analysis but their categorization far remains uncertain and unclassified (Rowley *et al.*, 2014; Matsui *et al.*, 2015, 2017; Zeng *et al.*, 2017).

During recent fieldwork in western Guangxi Province, China, we discovered a small tree frog that closely resembles to *G. jinggangensis*, *G. jinxiuensis* and *G. sapaensis*, but the morphological characters, advertisement calls and genetic data differed from these and all other rhacophorids in China and adjoining countries. We here describe these specimens as a new discovered species.

2. Materials and Methods

All specimens were collected and deposited at the Natural History Museum of Guangxi (NHMG). We recorded morphological data from the specimens after fixing them in 10% formalin and then stored in 75% ethanol. Measurements were taken with digital calipers to the nearest 0.1 mm. Measurements included snout-

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vent length (SVL); head length from tip of snout to rear of jaws (HL); head width at the commissure of the jaws (HW); snout length from tip of snout to the anterior corner of eye (SNT); diameter of the exposed portion of the eyeball (ED); interorbital distance (IOD); horizontal diameter of tympanum (TD); upper eyelid width, greatest width of upper eyelids (UEW); distance from anterior edge of tympanum to posterior corner of the eye (TED); internarial space (IN); distance from front of eye to nostril (EN); tibia length with the hindlimb flexed (TIB); tibia width (TW); forelimb length from tip of disk of finger III to axilla (FLL); hand length from the base of outer palmar tubercle to the tip of third finger (HAL); maximal diameter of disc of third finger (FTD_3); hindlimb length from tip of disk of fourth toe to groin (HLL); foot length from the base of inner metatarsal tubercle to the tip of fourth toe (FOL); and diameter of fourth toe tip, greatest diameter of disk on fourth toe (HTD_4). The webbing formula is given as proposed by Myers and Duellman (1982).

We obtained comparative morphological data for the 13 recognized species in the genus *Gracixalu* from the literature as discussed in Boulenger (1893), Bourret (1937), Hu (1978), Orlov and Nguyen (2004), Nguyen *et al.* (2008, 2013), Fei *et al.* (2010), Mo *et al.* (2013), Rowley *et al.* (2011, 2014), Matsui *et al.* (2015, 2017), and Zeng *et al.* (2017).

Advertisement calls were recorded with an ICD recorder (Sony ICD-TX50). The calls were recorded at a distance about 0.1-0.3 m and ambient temperatures were taken with a TP-2200 (A-volt). Calls were analyzed with Raven Pro 1.3 beta version (http://www.birds.cornell.edu/raven) with the default settings.

A preliminary phylogenetic analysis was also reconstructed based on a fragment of the mitochondrial gene 16S (~530 bp). We used the primers 16Sar and 16Sbr (Palumbi *et al.*, 1991) to amplify the fragment. Genomic DNA was extracted from tissue samples, using DNeasy tissue extraction kits (QIAGEN). PCR products were directly sequenced with an ABI 3730 automated DNA sequencer. Newly determined sequences were submitted for BLAST search in GenBank to ensure that the required sequences had been amplified (Altschul *et al.*, 1997), and then deposited in GenBank (MH117960-61). Eleven out of 13 species of *Gracixalus* were included in our phylogenetic analyses: *Gracixalus gracilipes* (Bourret, 1937), *Gracixalus jinggangensis* (Zeng *et al.*, 2017), *Gracixalus jinxiuensis* (Hu, 1978), *Gracixalus lumarius* (Rowley *et al.*, 2014), *Gracixalus nonggangensis* (Mo *et al.*, 2013), *Gracixalus quangi* (Rowley *et al.*, 2011), *Gracixalus quyeti* (Nguyen *et al.*, 2008), *Gracixalus sapaensis* (Matsui *et al.*, 2017), *Gracixalus seesom* (Matsui *et al.*, 2015), *Gracixalus supercornutus* (Orlov *et al.*, 2004), *Gracixalus waza* (Nguyen *et al.*, 2013). All but *G. supercornutus* came from the type localities. The sequences of *Gracixalus carinensis* (Boulenger, 1893) and *Gracixalus medogensis* (Ye and Hu, 1984) were unavailable. In addition, for *G. jinxiuensis* group, we also chose specimens from non-type localities, including Hunan and Yunnan Provinces, China, Lai Chau, Nghe An, Lao Cai, Vietnam, and Houapan, Laos (Table 1).

Phylogenetic analyses were done using Bayesian inference (BI) implemented in MrBayes 3.12 (Ronquist and Huelsenbeck, 2003) and maximum likelihood (ML) on the RAxML Web server (http://phylobench.vital-it.ch/raxml-bb/; Stamatakis *et al.*, 2008). The optimal nucleotide substitution model (GTR+I+G) was selected based on the Akaike Information Criterion (Posada and Buckley, 2004) as implemented in MrModeltest 2.3 (Nylander, 2004). Two independent runs with four Markov Chain Monte Carlo simulations were performed for 10 million iterations and sampled every 1000th iteration and the first 25% of samples were discarded as burn-in. *Rhacophorus borneensis* (Matsui *et al.*, 2013) and *Kurixalus odontotarsus* (Ye and Fei, 1993) were used as outgroups following Matsui *et al.* (2017). Uncorrected pairwise (*p*-distance) sequence divergence was calculated using MEGA version 7 (Tamura *et al.*, 2016).

3. Results

***Gracixalus tianlinensis* sp. nov.**

Holotype NHMG1706002 (Figure 1 A-C), adult male, from Cenwanglaoshan National Nature Reserve, Tianlin County, Guangxi, China (24.4883º N, 106.3947º E, 1858 m), collected by Yunming Mo, Xiaowen Liao and Shichu Zhou on 8 June 2017.

Paratypes NHMG1705009-11, 1706001-2, 1706006-8, adult males, and 1705008, 1706003-5, adult females from the same site as the holotype, collected by Yunming Mo, Shichu Zhou, Zhiming Xie and Xueqiang Lei, on 23 May and 8 June 2017.

Diagnosis *G. tianlinensis* sp. nov. is assigned to the genus *Gracixalus* based on molecular data and morphological characters. Morphologically, the new species presents an intercalary cartilage between the terminal and penultimate phalanges of digits, tips of digits expanded into large disks with circummarginal grooves and also the vomerine teeth were absent with the pupil being horizontal. *Gracixalus tianlinensis* sp. nov. is distinguished from all

Table 1 Samples and sequences used in this study. TL= type locality. The new determined sequences in this study were shown in italic bold. Generic allocation according to Frost (2017). Voucher abbreviations: AMS: Australian Museum; BORN: BORNEENSIS, University Malaysia, Sabah; CIB: Chengdu Institute of Biology; CUMZ: Chulalongkorn University Museum of Natural History; IEBR: Institute of Ecology and Biological Resources, Hanoi; KIZ: Kunmin Institute of Zoology; KUHE: Graduate School of Human and Environmental Studies, Kyoto University; MNHN: Muséum national d'Histoire naturelle, Paris; NHMG: Natural History Museum of Guangxi; SYS: Sun Yat-sen University; VNMN: Vietnam National Museum of Nature; VNUH: Vietnam National University, Hanoi; ZFMK; Zoologisches Forschungs-museum Alexander Koenig.

ID	Species	Voucher no.	Locality	Reference	GenBank no.
1	*G. gracilipes*	MNHN 1999.592	Vietnam, Lao Cai Province (TL)	Rowley *et al.*, 2011	AY880504
2	*G. gracilipes*	AMNH A163897	Vietnam, Ha Giang Province	Rowley *et al.*, 2011	DQ283051
3	*G. gracilipes*	KIZ060821196	China, Yunnan Province	Rowley *et al.*, 2011	EF564523
4	*G. jinggangensis*	SYS a003170 (paratype)	China: Mt. Jinggang, Jiangxi	Zeng *et al.*, 2017	KY624586
5	*G. jinggangensis*	SYS a003186 (paratype)	China: Mt. Jinggang, Jiangxi	Zeng *et al.*, 2017	KY624587
6	*G. jinxiuensis*	KIZ 061210YP	China, Jinxiu County, Guangxi (TL)	Li *et al.*, 2011	EU215525
7	*G. jinxiuensis*	SYS a002182	China, Jinxiu County, Guangxi (TL)	Zeng *et al.*, 2017	KY624584
8	*G. jinxiuensis*	SYS a002183	China, Jinxiu County, Guangxi (TL)	Zeng *et al.*, 2017	KY624585
9	*G. jinxiuensis*	KIZ060821013	China, Jinxiu County, Guangxi (TL)	Yu *et al.*, 2009	EF564524
10	*G. jinxiuensis*	KIZ 060821126	China, Yunnan Province	Li *et al.*, 2009	EF564525
11	*G. jinxiuensis*	CIB HN201108200	China, Hunan Province	Matsui *et al.*, 2015	LC011936
12	*G. jinxiuensis*	KUHE 32453	Laos, Houapan	Matsui *et al.*, 2015	LC011937
13	*G. jinxiuensis*	AMS R 173454	Vietnam, Nghe An Province	Rowley *et al.*, 2011	JN862547
14	*G. lurnarius*	AMS R 176202 (holotype)	Vietnam, Kon Tum (TL)	Rowley *et al.*, 2014	KF918412
15	*G. nonggangensis*	NHMG200910010 (paratype)	China, Guangxi Province (TL)	Mo *et al.*, 2013	JX841318
16	*G. nonggangensis*	NHMG1005046 (paratype)	China, Guangxi Province (TL)	Mo *et al.*, 2013	JX841320
17	*G. quangi*	AMS R 173410 (holotype)	Vietnam, Nghe An Province (TL)	Rowley *et al.*, 2011	JN862537
18	*G. quangi*	AMS R 173411 (paratype)	Vietnam, Nghe An Province (TL)	Rowley *et al.*, 2011	JN862538
19	*G. quyeti*	VNUH160706 (paratype)	Vietnam: Quang Binh (TL)	Nguyen *et al.*, 2008	EU871428
20	*G. quyeti*	ZFMK 82999 (holotype)	Vietnam: Quang Binh (TL)	Nguyen *et al.*, 2008	EU871429
21	*G. sapaensis*	VNMN 4211	Vietnam, Lao Cai (TL)	Matsui *et al.*, 2017	LC140971
22	*G. sapaensis*	VNMN 4212	Vietnam, Lao Cai (TL)	Matsui *et al.*, 2017	LC140972
23	*G. sapaensis*	VNMN 4358	Vietnam, Lao Cai (TL)	Matsui *et al.*, 2017	LC140973
24	*G. sapaensis*	MNHN 1999.5964 (paratype)	Vietnam, Lao Cai (TL)	Matsui *et al.*, 2017	LC140969
25	*G. sapaensis*	MNHN 1999.5966 (paratype)	Vietnam, Lao Cai (TL)	Matsui *et al.*, 2017	LC140970
26	*G. sapaensis*	IEBR 2351	Vietnam, Lai Chau Province	Matsui *et al.*, 2017	EU871425
27	*G. sapaensis*	IEBR 2352	Vietnam, Lai Chau Province	Matsui *et al.*, 2017	EU871426
28	*G. seesorn*	KUHE 35084 (holotype)	Thailand, Kanchanaburi (TL)	Matsui *et al.*, 2015	LC011932
29	*G. seesorn*	KUHE 35088 (paratype)	Thailand, Kanchanaburi (TL)	Matsui *et al.*, 2015	LC011933
30	*G. seesorn*	KUHE 35089 (paratype)	Thailand, Kanchanaburi (TL)	Matsui *et al.*, 2015	LC011934
31	*G. seesorn*	CUMZ K1856 (paratype)	Thailand, Kanchanaburi (TL)	Matsui *et al.*, 2015	LC011935
32	*G. supercornutus*	AMS 8173395	Vietnam: Kon Tum Province	Rowley *et al.*, 2011	JN862542
33	*G. supercornutus*	AMS R 173887	Vietnam, Kon Tum Province	Rowley *et al.*, 2011	JN862545
34	***G. tianlinensis* sp. nov.**	NHMG1705015 (paratype)	China, Guangxi Province (TL)	This study	MH117960
35	***G. tianlinensis* sp. nov.**	NHMG1705016 (paratype)	China, Guangxi Province (TL)	This study	MH117961
36	*G. waza*	IEBR A.2012.2 (holotype)	Vietnam, Cao Bang (TL)	Nguyen *et al.*, 2013	JX896681
37	*G. waza*	VNMN A.2012.2 (paratype)	Vietnam, Cao Bang (TL)	Nguyen *et al.*, 2013	JX896684
38	*Gracixalus* sp.	CIB 03320Rao	China, Yunnan Province	Li *et al.*, 2009	GQ285669
39					
40	*Kurixalus odontotarsus*	KIZ 201307071	China, Yunnan Province	Nguyen *et al.*, 2014	AB933303
41	*Rhacophorus borneensis*	BORN 22410	Malaysia, Sabah	Matsui *et al.*, 2013	AB781693

other rhacophorids by a combination of (1) SVL 30.3-35.9 mm in male, 35.6-38.7 mm in female, (2) head length less than head width, (3) vomerine teeth absent, (4) supratympanic fold distinct, (5) axilla and posterior surface of flanks pale yellow, (6) nuptial pads distinct on the first finger and slightly visible on the second finger, (7) dorsum brown to beige, with an inverse Y-shaped dark brown marking, (8) single subgular vocal sac.

Description of holotype Body dorsoventrally compressed; head length less than head width (HL/HW=0.9); snout rounded in dorsal view and in profile, projecting beyond margin of the lower jaw; canthus rostralis distinct and rounded; loreal region sloping and concave; interorbital region flat; nostril oval, laterally positioned, slightly protuberant, nearer tip of snout than eye; internarial distance greater than eye to nostril

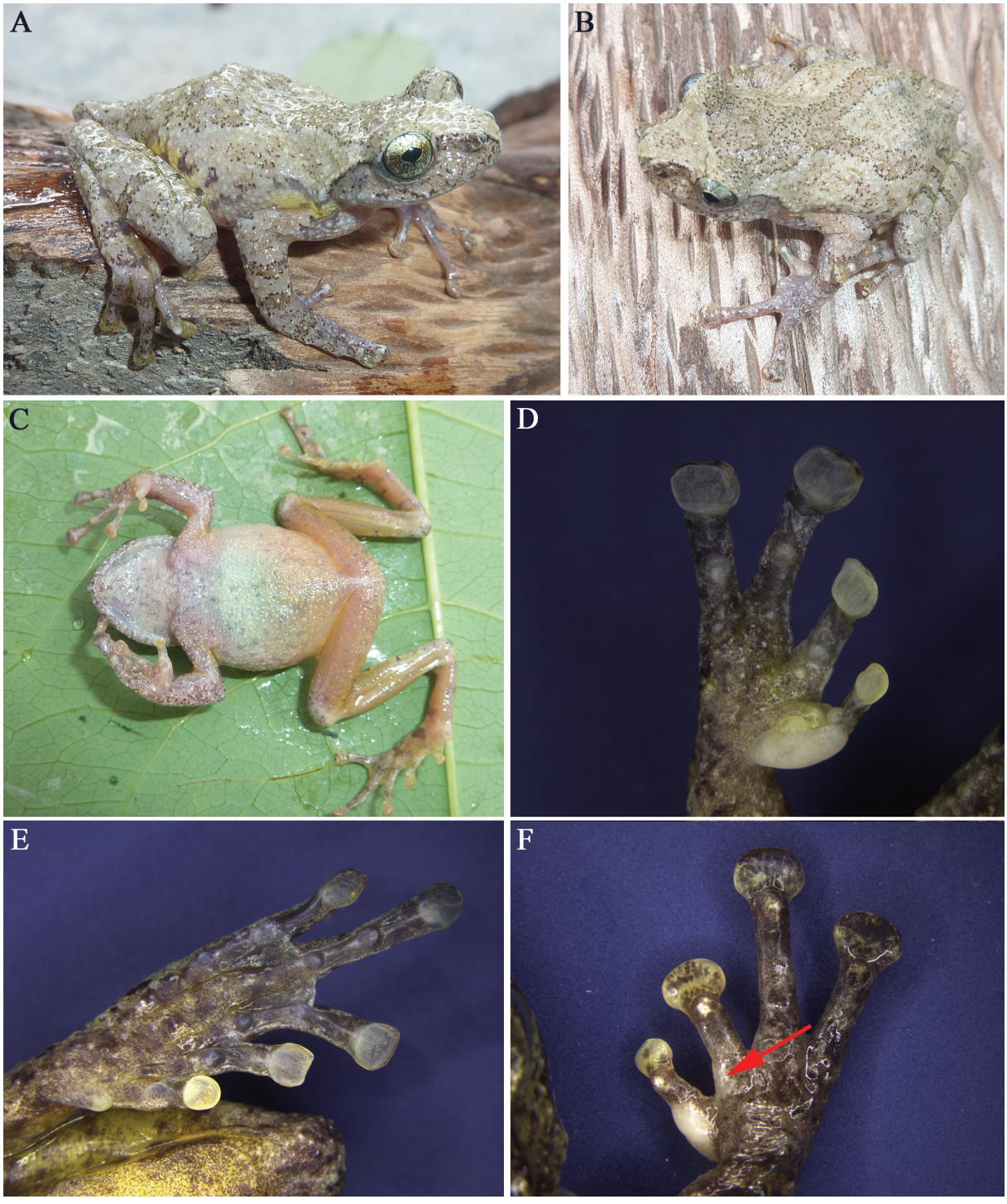

Figure 1 (A) Dorsolateral, (B) dorsal and (C) ventral view of the holotype in life, (D) ventral view of the hand and (E) ventral view of the foot of the holotype, (F) showing nuptial pads on Finger I and Finger II.

distance (IN/EN=1.5); pupil horizontal; eye diameter greater than eye to nostril distance (ED/EN=1.5); interorbital width greater than upper eyelid width (IOD/UEW=1.2); tympanic diameter less than eye diameter (TD/ED=0.5); supratympanic fold distinct and extending to axilla; vomerine teeth absent; choanae small and oval; tongue attached anteriorly, deeply notched posteriorly; external single subgular vocal sac.

Forelimb relatively robust, relative length of fingers I < II < IV < III; tips of all fingers with well-developed disks with horizontal circummarginal grooves, disks relatively wide compared to finger width, third finger

disk width greater than tympanic diameter (FTD_3/TD=1.2); fingers webbing absent; subarticular tubercles prominent, rounded, formula 1, 1, 2, 2; accessory palmar tubercles indistinct; nuptial pads present on posterolateral surface of Finger I and on basis of Finger II; nuptial pads of Finger I strongly dilated, and of Finger II small and slightly dilated. Relative length of toes I < I < III < V < IV; tips of toes disks with distinct circummarginal grooves; disks smaller than those of fingers; toes moderately webbed, webbing formula: I 1–1^- II 1^+–1^+ III 2^+–2 IV 2^+–2 V; subarticular tubercles rounded, distinct, formula 1, 1, 2, 3, 2; inner metatarsal tubercle oval, elongated; outer metatarsal tubercle and supernumerary tubercle absent (Figure 1 D-F). Dorsal surface of head, body and limbs rough, scattered tubercles, ventral surface of limbs smooth; chest, throat and belly granular; tarsal fold absent.

Measurements of holotype (in mm) SVL 33.6, HL 11.3, HW 12.3, SNT 5.3, ED 4.2, IOD 3.9, TD 2.0, UEW 3.2, TED 0.9, IN 4.2, EN 2.8, TIB 16.1, TW 5.3, FLL 17.5, HAL 11.0, FTD_3 2.4, HLL 50.1, FOL 15.8, HTD_4 2.1.

Color of holotype in life Dorsal surface was found to be ranging from brown to beige, with an inverse Y-shaped dark brown marking across back, covering interorbital region and eyelids, bifurcating into two branches on the shoulder, and reaching the posterior of the back. Axilla and posterior surface of flanks pale yellow. Dorsal surface of arms with two brown transverse bands, and dorsal surface of limbs with three brown transverse bands. Ventral surface of the throat, chest gray with small dark specks. Belly creamy white. Ventral surface of thighs half-transparent creamy yellow. Iris bronze with a network of fine brown reticulations; pupil black.

Color of holotype in preservative Dorsal surface of head, body and limbs brown; Y-shaped marking and transverse bands black; ventral surface of chest and belly pale white and throat gray with variable brown marbling.

Variation Measurements of the type series are shown in Table 2. All males have nuptial pads on Finger I and Finger II. On Finger I, the nuptial pads are strongly dilated, but small or just rudimentary on Finger II in some specimens. In life, dorsal coloration varied among individuals and within individuals over time and may be brown to beige or dark brown. The ventral surface of the throat varied from gray to brown.

Etymology This species is named after the locality in which it was collected. The suggested English name is Tianlin small tree frog (田林纤树蛙 in Chinese).

Advertisement call Ambient air temperature was 18.0 °C when the male calls were recorded. The male advertisement call of the new species consists of one note with two pules per note. The interval of calls varies from 2 to 5s. The dominant frequency spectrum of the calls lays between 2.0 and 3.0 kHz, and harmonic presents at 4.5-5.5 kHz (Figure 2).

Molecular relationships Our preliminary phylogenetic trees were like Rowley *et al.* (2014), Zeng *et al.* (2017) and Matsui *et al.* (2017) with the exception of the generic placement of *G. lumarius*. In our results, the generic placement of *G. lumarius* was treated as Clade III. Then,

Table 2 Measurements (mm) of *Gracixalus tianlinensis* sp. nov. Abbreviations defined in text.

Character	Male		Female	
	Range	Mean±SD	Range	Mean±SD
SVL	30.3–35.9	32.5±1.8	35.6–38.7	36.6±2.5
HL	10.2–12.0	11.3±0.6	12.6–13.7	13.1±1.0
HW	11.6–13.0	12.4±0.5	13.2–14.7	14.0±1.0
SNT	4.9–5.3	5.1±0.2	5.5–5.8	5.7±0.3
ED	3.9–4.4	4.2±0.2	4.4–4.7	4.6±0.2
IOD	3.5–3.9	3.7±0.1	3.8–4.6	4.2±0.3
TD	1.6–1.9	1.8±0.1	2.3–2.4	2.3±0.1
UEW	2.6–3.5	3.2±0.3	3.3–3.4	3.4±0.2
TED	0.8–1.2	1.0±0.2	1.2–1.3	1.3±0.1
IN	3.7–4.2	4.0±0.2	4.2–4.4	4.3±0.2
EN	2.7–3.2	2.8±0.2	3.3–3.8	3.5±0.4
TIB	16.1–18.8	16.9±0.9	17.6–18.9	18.4±1.1
TW	4.1–5.3	4.7±0.3	4.7–5.5	5.2±0.4
FLL	17.1–20.4	18±1.1	19.7–20.8	20.1±1.4
HAL	10.1–12.2	11.1±0.6	11.9–13.5	12.6±1.0
FTD3	2.0–2.5	2.2±0.2	2.4–3.1	2.6±0.3
HLL	48.8–56.6	51.3±2.4	54.4–58.9	56.6±3.4
FOL	15.1–17.8	15.7±0.9	16.7–18.4	17.5±1.2
HTD4	1.5–2.2	1.9±0.2	1.8–2.2	2.0±0.2

we identified three supported clades within species that is currently proposed within the genus *Gracixalus*, which is similar to Rowley *et al.* (2014), Zeng *et al.* (2017) and Matsui *et al.* (2017). Clade I consists of *G. gracilipes*, *G. quangi*, *G. quyeti*, *G. seesom* and *G. supercornutus.* Clade II consists of *G. jinggangensis*, *G. jinxiuensis* group, *G. nonggangensis*, *G. sapaensis*, *Gracixalus* sp., *G. waza* and *G. tianlinensis* sp. nov. *Gracixalus tianlinensis* sp. nov. is sister to *G. sapaensis* with well-supported values (BP=0.99; PP=96) (Figure 3). Uncorrected pairwise distance (*p*-distance) between *G. tianlinensis* sp. nov. and *G. sapaensis* is 2.3-3.2%. *Gracixalus jinxiuensis* group did not form a monophyletic group. *Gracixalus jinxiuensis* (TL) is close to *G. jinggangensis*, and both are close to *G. jinxiuensis* (Hunan) with a large distance (*p*-distance=5.8%). Among *G. jinxiuensis* (China-Laos-Vietnam) group, three sequences share one haplotype (~450 bp) and are close to *Gracixalus* sp. with a large distance (*p*-distance=5.0-5.1%) (Table 3).

Comparisons *Gracixalus tianlinensis* sp. nov. can be distinguished from most of the species of *Gracixalus* by having a brown to beige dorsum with an inverse Y-shaped dark brown marking, rounded snout in dorsal view and in profile, axilla and posterior surface of flanks pale yellow, distinct nuptial pads on Finger I and slightly visible on basis of Finger II, with the exception of *G. jinggangensis*, *G. jinxiuensis* and *G. sapaensis*. Considering advertisement calls, *G. tianlinensis* sp. nov. just has one note and the dominant frequency spectrum of the calls lay between 2.5 and 3.1 kHz. But *G. jinggangensis* and *G. jinxiuensis* have more than two notes of the advertisement calls (Hu *et al*., 1978; Zeng *et al*., 2017). Morphologically, *G. tianlinensis* sp. nov. differs from *G. jinggangensis* by having axilla and posterior surface of flanks pale yellow (*vs*. pale yellow absent in *G. jinggangensis*); finger webbing absent (*vs*. finger webbing rudimentary in *G. jinggangensis*); temporal region and corner of the mouth without large tubercles (*vs.* densely covered with large tubercles in *G. jinggangensis*); webbing formula, I 1–1^{-} II 1^{+}–1^{+} III 2^{+}–2 IV 2^{+}–2 V (*vs.* I 2–2^{+} II 1^{+}–3 III 2–3 IV 3–2 V in *G. jinggangensis*). *Gracixalus tianlinensis* sp. nov. differs from *G. jinxiuensis* by having a larger size in adults (SVL 30.3-35.9 mm in male and 35.7-38.7 mm in female *vs*. 24.2-26.3 mm in males and 28.0-29.2 mm in females in *G. jinxiuensis*); nuptial pads on Finger I and Finger II of males (*vs.* only on Finger I in *G. jinxiuensis*); axilla and posterior surface of flanks pale yellow (*vs.* pale yellow absent in *G. jinxiuensis*). *Gracixalus sapaensis*

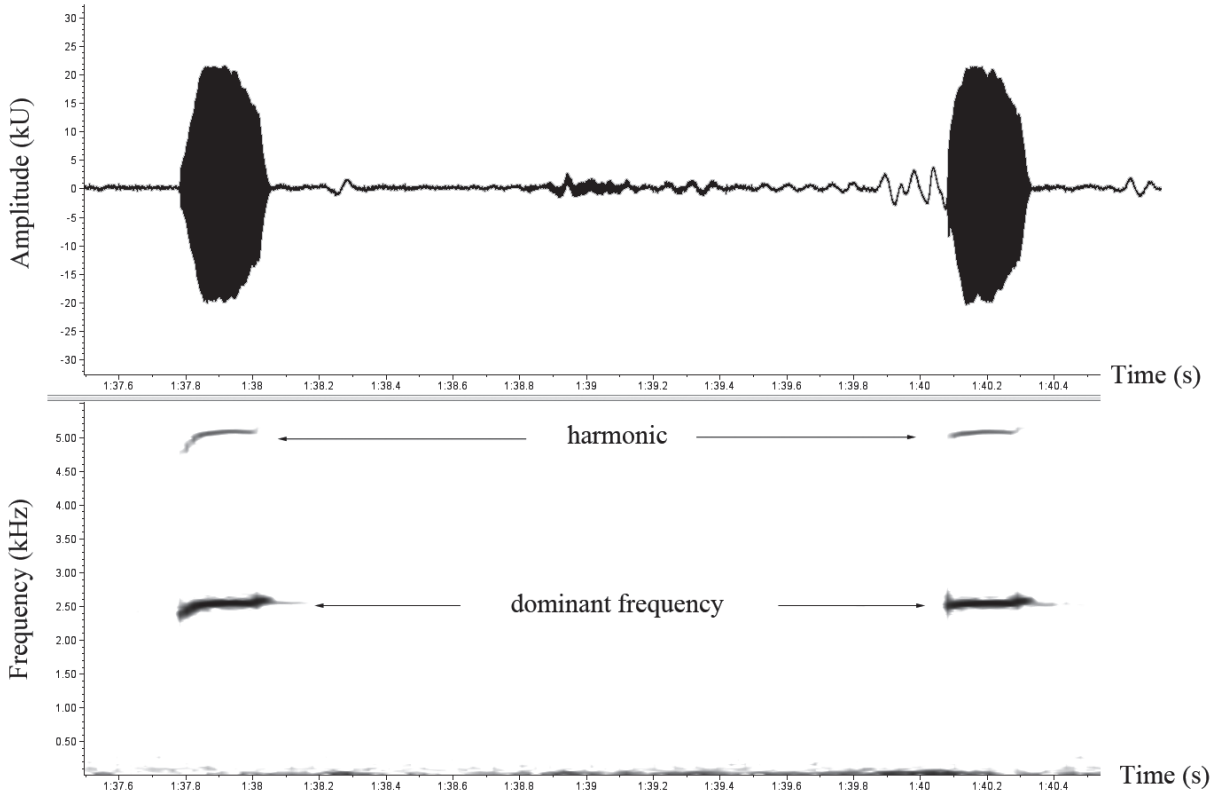

Figure 2 Oscillogram and corresponding audiospectrogram of two advertisement calls of a paratypic male *G. tianlinensis* sp. nov. (NHMG1706006). Ambient air temperature was 18.0 °C.

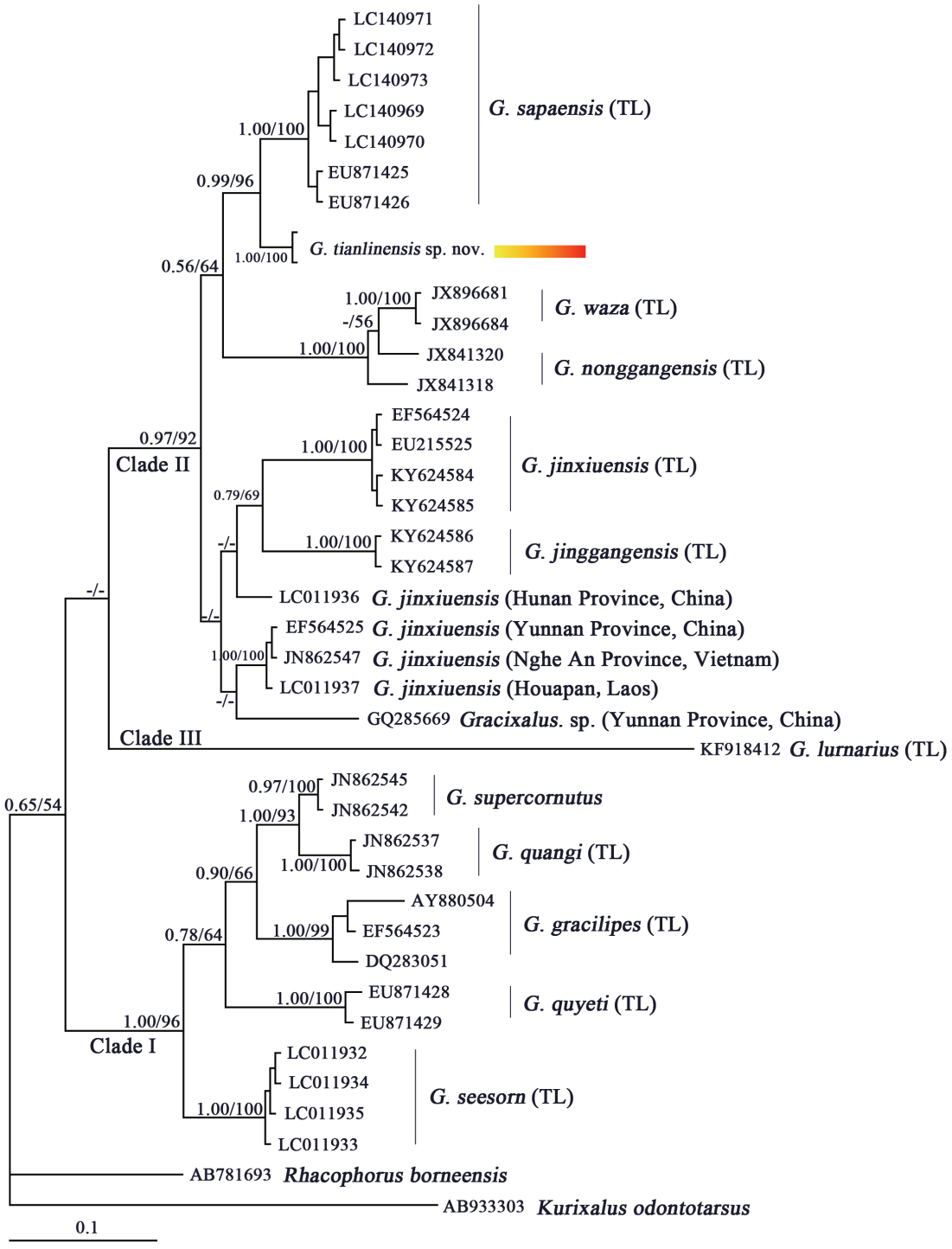

Figure 3 Bayesian inference tree reconstructed from 16S rRNA mitochondrial gene with *Kurixalus odontotarsus* and *Rhacophorus borneensis* as outgroups. Numbers above branches represent bootstrap supports for Bayesian posterior probabilities (BPP) (> 0.5 retained)/ bootstrap support for maximum likelihood analyses (> 50 retained); '-' represents BPP and bootstrap proportions lower than 50% and 50%, respectively.

has dermal fringe along fifth toe, from tip of toe to base of metatarsal; nuptial spines absent on Finger I; a pair of long vocal slits on both sides of mouth floor well anterior to jaw commissure, dorsal surface golden ochre with a darker brownish marking on upper eyelid and across back, forming X marking, webbing yellowish; ventral surface of throat, chest, belly, and forelimb light yellow, hindlimb darker yellow; third finger disk width less than

Table 3 Uncorrected *p*-distances (in %) in the genus *Gracixalus* based on mitochondrial 16S rRNA sequences (~530 bp). TL = type locality, C-L-V = China-Laos-Vietnam.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	*G. sapaensis*	0–0.7														
2	*G. tianlinensis* sp. nov.	2.3–3.2	0													
3	*G. waza*	6.4–7.5	6.4	0												
4	*G. nonggangensis*	7.0–8.3	7	2.5–2.7	2.7											
5	*G. jinggangensis*	6.2–6.5	5.6	7.5	7.1–7.5	0										
6	*G. jinxiuensis* (TL)	6.2–7.6	5.8	7.5–8.0	7.5–8.9	6.9	0									
7	*G. jinxiuensis* (Hunan)	4.6–4.8	4.8	7	6.2–7.7	5.3	5.8	0								
8	*G. jinxiuensis* (C-L-V)	4.3–5.0	4.3–4.4	5.7–6.6	5.5–7.2	5	5.8–6.2	2.2	0							
9	*Gracixalus* sp.	6.0–6.8	6.5	9.3	8.5–8.9	7.7	7.3–7.5	5.5	5.0–5.1	0						
10	*G. lumarius*	15.3–16.7	15.2	16.6	16.0–16.5	14.9	16.0–16.5	14.7	14.3–15.1	14.6	0					
11	*G. supercornutus*	10.4–11.5	10.7–10.9	12.6–12.8	11.5–12.2	9.3–9.5	10.9–11.1	9.4–9.7	9.0–10.1	10.5–10.7	15.6–15.8	0.2				
12	*G. quangi*	9.0–9.7	9.6	11.9	10.8–11.3	8	9.9–10.2	8.5	8.4–8.5	10	14.7	2..5–2.7	0			
13	*G. gracilipes*	10.1–11.8	10.2–10.9	12.6–13.2	11.9–13.1	10.7–11.6	10.9–11.9	10.2–11.1	10.2–11.5	11.1–12.9	14.9–15.6	6.1–7.5	4.8–6.2	1.3–3.0		
14	*G. quyeti*	9.8–11.4	9.4–10.0	12.0–12.2	11.3–12.0	10.7–11.4	9.8–10.5	10.2–10.4	9.8–10.8	10.6–11.2	13.8–14.3	5.7–6.1	5.3–5.9	6.5–8.7	0.6	
15	*G. seesom*	8.5–9.1	7.8	10.4	10.2–10.4	9.9	9.7	8.7	9	10.1	16.4	6.7–6.9	5.8	6.0–6.9	7.1–7.8	0

tympanic diameter; webbing formula: I $2–2^{2/3}$ II $1^{1/2}–3$ III $2–3^{1/3}$ IV $2^{2/3}–1^{1/2}$ V; inner palmar tubercle flat, outer palmar tubercle divided into two, and iris golden with greenish reflection (*vs*. dermal fringe absent, external single subgular vocal sac, dorsal surface brown to beige, webbing gray, ventral surface of the throat, chest gray with small dark specks, belly creamy white, accessory palmar tubercles indistinct, third finger disk width greater than tympanic diameter (FTD_3/TD=1.2), webbing formula: I $1–1^-$ II $1^+–1^+$ III $2^+–2$ IV $2^+–2$ V, iris bronze with a network of fine brown reticulations, and nuptial pads on Finger I and Finger II in *G. tianlinensis* sp. nov.).

Gracixalus tianlinensis sp. nov. differs from *G. carinensis* as it has SNT longer than ED (*vs*. SNT less than ED in *G. carinensis*), ventral surface of chest and belly pale white and throat gray with variable brown marbling (*vs*. immaculate white in *G. carinensis*), nuptial pads on Finger I and Finger II (*vs*. on Finger I in *G. carinensis*) (Boulenger, 1893; Zeng *et al.*, 2017; Matsui *et al.*, 2017). *Gracixalus nonggangensis* and *G. waza* differ from *G. tianlinensis* sp. nov. by having yellow-green dorsum, supratympanic fold indistinct (*vs*. brown dorsum and supratympanic fold distinct in the new species), dorsal skin smooth (*vs*. rough in the new species), outer palmar tubercle divided into two (*vs*. palmar tubercles indistinct in the new species), and nuptial pads on Finger I (*vs*. on Finger I and Finger II in the new species). *Gracixalus gracilipes*, *G. quangi*, *G. quyeti*, and *G. supercornutus* differ from *G. tianlinensis* sp. nov. by having a greenish dorsum and spines on upper eyelid present (*vs*. brown to beige dorsum and spines on upper eyelid absent in the new species). *Gracixalus gracilipes*, *G. quangi*, and *G. supercornutus* have a triangular pointed snout (*vs*. snout rounded in the new species). Furthermore, *G. quyeti*, and *G. supercornutus* have a tibiotarsal projection (*vs*. absent in the new species). *Gracixalus quangi* differs from *G. tianlinensis* sp. nov. by having the greenish translucent skin, triangularly pointed snout and the presence of a tibiotarsal projection (*vs*. brown dorsum, rounded snout and tibiotarsal projection absent in the new species). *Gracixalus lumarius* differs from *G. tianlinensis* sp. nov. by having venter pink (*vs*. creamy white in the new species), supratympanic fold indistinct (*vs*. supratympanic fold distinct and extending to axilla in the new species), dorsum with distinctive white conical tubercles in adult males (*vs*. absent white conical tubercles in the new species), nuptial pads on inner surface of the prepollex (*vs*. nuptial pads on Finger I and Finger II in the new species). *Gracixalus medogensis* has smaller body size, SVL 26.5 mm in male; dorsal skin smooth; nuptial pads

on the first fingers in male present (*vs.* SVL, 30.3-35.9 mm in male and 35.7-38.7 mm in female; dorsal skin rough; nuptial pads on Finger I and Finger II in male present in new species). *Gracixalus tianlinensis* sp. nov. differs from *G. seesom* by having a larger SVL, 30.3-35.9 mm in male and 35.7-38.7 mm in female (*vs.* 21.6-23.0 mm in males, 23.2-25.4 mm in females in *G. seesom*), a single subgular vocal sac in male (*vs.* a pair of vocal slits in *G. seesom*), rounded snout (*vs.* triangular snout in *G. seesom*), brown dorsum with an inverse Y-shade marking (*vs.* light yellowish brown dorsum without an inverse Y-shade marking in *G. seesom*), nuptial pads on Finger I and Finger II (*vs.* nuptial pads absent in *G. seesom*).

4. Discussion

Given the morphological distinctness, advertisement calls, and genetic evidence, we described *G. tianlinensis*

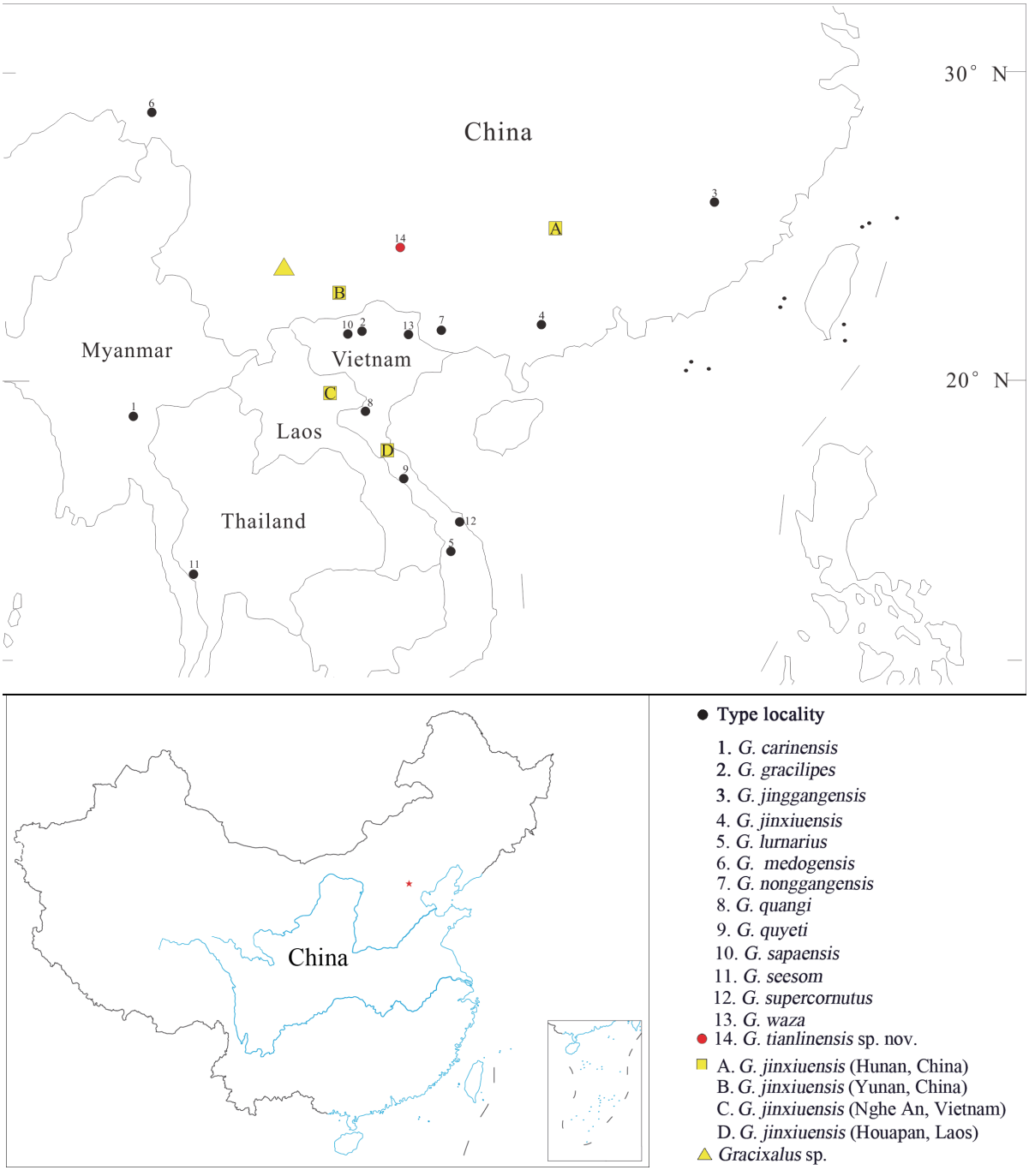

Figure 4 Map of the type localities of *Gracixalus*.

sp. nov. as a new member of *Gracixalus*. Although *G. tianlinensis* sp. nov. is similar to *G. jinggangensis* and *G. jinxiuensis*, their advertisement calls and genetic variation are distinct from each other (Table 3, Figure 2 and Figure 3).

The correct identification of the taxa studied is essential for phylogenetic hypothesis. Matsui *et al.* (2017) pointed out that *G. jinxiuensis* (GenBank no. EU871425-26, from Lai Chau, Vietnam), *G. carinensis* (LC011938-39, LC140969-70, Lao Cai, Vietnam) and *Aquixalus odontotasrus* (AY880503, Lao Cai, Vietnam) were mis-identified. Above three species were assigned to *G. sapaensis* based on their molecular and morphological data. In our phylogenetic analyses, most of sequences either were holotype and paratype, or from topotype, which ensured the correct identification. In our preliminary results, we detected three distinct clades, which were similar to Rowley *et al.* (2014) and Matsui *et al.* (2015, 2017) (Figure 3). Clade III just included *G. lumarius* with weakly supported values. Position of *G. lumarius* was enigmatic and needs to be tested further. In Clade I, five species, *G. gracilipes*, *G. quangi*, *G. quyeti*, *G. supercornutus*, *G. seesom* form five evolutionary branches with relatively robust values (Figure 3). Interestingly, in Clade II, the individuals of *G. jinxiuensis* formed the non-monophyly. *Gracixalus jinxiuensis* (TL) formed a monophyly and was close to *G. jinggangensis* with a large distance (*p*-distance=6.9%). The genetic distance between *G. jinxiuensis* (TL) and *G. jinxiuensis* (Hunan) reached 5.8%, *G. jinxiuensis* (TL) and *G. jinxiuensis* (China-Laos-Vietnam) is 5.8-6.2% (Table 3). Considering relative large genetic variation and phylogenetic analyses, we suggest that *G. jinxiuensis* (Hunan) and *G. jinxiuensis* (China-Laos-Vietnam) are two unknown members of *Gracixalus*. The genetic distance between *Gracixalus* sp. (Yunnan, GQ285669) and *G. jinxiuensis* (TL), *G. jinxiuensis* (Hunan) and *G. jinxiuensis* (China-Laos-Vietnam), all are larger than 5.0% (Table 3). Hence, we suggest that *Gracixalus* sp. (Yunnan, GQ285669) is an unknown member of *Gracixalus* too. Figure 4 showed most type locality of the genus *Gracixalus*.

So far, *G. tianlinensis* sp. nov. is only found in Cenwanglaoshan National Nature Reserve and restricted to high-elevation evergreen forest with montane bamboo. More distribution of the new species is unknown.

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